Detection and quantification of Hepatitis C Virus using the new Aptima HCV Quant Dx assay in the fully automated Panther® System compared to the Abbott Realtime HCV assay.

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ABSTRACT

Background: Hepatitis C Virus (HCV) continues to be an important health concern worldwide. Different therapeutic methods are now available for the treatment of HCV infection with good results. Determining the viral load of patients under treatment is now the standard of care for monitoring the response to these treatments. There are different commercially available assays used to detect and quantify HCV RNA in serum and plasma specimens. The objective of this study was to compare the Aptima HCV Quant Dx assay, recently released by Hologic, Inc®, with the Abbott Molecular Realtime HCV assay. The Aptima HCV Quant Dx assay is a real-time transcription-mediated amplification (TMA) test, run in the Panther System (Hologic) used for confirmation of diagnosis and monitoring of HCV RNA. The Abbott Realtime HCV assay is an RT-PCR test run on the automated m2000 system (Abbott Diagnostics).

Methods: Sixty plasma specimens, twenty negatives and forty positives for HCV were included in this study. All sixty specimens were used to test the qualitative performance and thirty of them, with known viral loads, were used to test the quantitative performance. All these specimens had been previously tested on the Abbott m2000 platform. The specimens were assayed using the Aptima HCV Quant Dx Assay on the Panther System following the manufacturer instructions. Specificity of the new assay was tested using twenty HCV negative specimens, some of which were positive for Cytomegalovirus (CMV) and Human Immunodeficiency Virus (HIV). Precision was tested using a known HCV positive specimen repeated twelve times in different runs. Results obtained from specimens tested in both instruments were compared using the EP Evaluator program.

Results: The EP Evaluator software was used to determine whether the methods are equivalent within a total allowable error of 1 $\log_{10}$ IU/mL. Thirty specimens with known HCV genotypes 1a, 1b, 2b, and 3a were compared over a range of 1.11 to 6.98 $\log_{10}$ IU/mL. The test passed with 98.3 % agreement. One specimen with low viral load was negative on the Abbott instrument and positive on the Panther system. This could be explained because the Aptima HCV Quant Dx Assay has a lower detection limit (<3.9 IU/mL) than the Abbott System (<12 IU/mL) in plasma specimens. The difference between the two methods was within allowable error. The average error index was 0.12 with a range of -0.41 and 0.54. The coefficient of correlation (R) between both methods was 0.9951. For the precision study, the EP Evaluator results showed a mean of 3.861 $\log_{10}$ IU/mL with a standard deviation of 0.047. This value was within the 2 SD range (3.767-3.954).

Conclusions: We can conclude that the Aptima HCV Quant Dx assay is a highly sensitive, accurate, and reproducible assay with a performance equal to that of the Abbott Realtime HCV assay. The Aptima HCV Quant Dx assay is a faster and more efficient test than the latter. This is helpful in the lab setting because it reduces hands on time needed to set up the test and allows for shorter wait time for results.

METHODS & PROCEDURES

Abbott m-2000 and Panther Hologic platforms were used to compare the agreement between both technologies to detect and quantify HCV RNA in plasma specimens.

Abbott m2000

Panther Hologic

Parameters	Abbott m2000	Panther Hologic
Specimen volume	1.1 mL	0.7 mL
Sample matrix	Serum or EDTA plasma	Serum or plasma (EDTA, ACD and PPT tubes).
Technology	RT-PCR	Real Time Transcription-Mediated Amplification (TMA)
Purpose of use	Quantification	Detection and Quantification
Turn around time	6 hrs	2.5 hrs to first result

METHODS & PROCEDURES CONT.

- ❑ In this study we included a total of sixty frozen specimens, twenty negatives and forty positives from HCV-infected patients, previously tested on the Abbott m2000 platform.
- ❑ All sixty specimens were used to test the qualitative performance and thirty of them, with known viral loads, were used to test the quantitative performance.
- ❑ The specimens were assayed using the Aptima HCV Quant Dx Assay on the Panther System following the manufacturer instructions.
- ❑ Specificity of the new assay was tested using twenty HCV negative specimens, some of which were positive for Cytomegalovirus (CMV) and Human Immunodeficiency Virus (HIV).
- ❑ Precision was tested using a known HCV positive specimen repeated twelve times in different runs and different days.
- ❑ Results obtained from specimens tested in both instruments were compared using the EP Evaluator program.

RESULTS

Method Comparison

A total of thirty specimens with known HCV viral loads and genotypes, where used to test the quantitative performance of Aptima HCV Quant Dx Assay over the range of 1.11 to 6.98 $\log_{10}$ IU/mL. Figures 1 and 2 show a high concordance between the two assays, with a difference within the allowable error (1 $\log_{10}$ IU/mL). The largest Error Index occurred at the concentration of 6.98 $\log_{10}$ IU/mL and the average error index was 0.12 with a range of -0.41 and 0.54. The coefficient of correlation (R) between both methods was 0.9951.

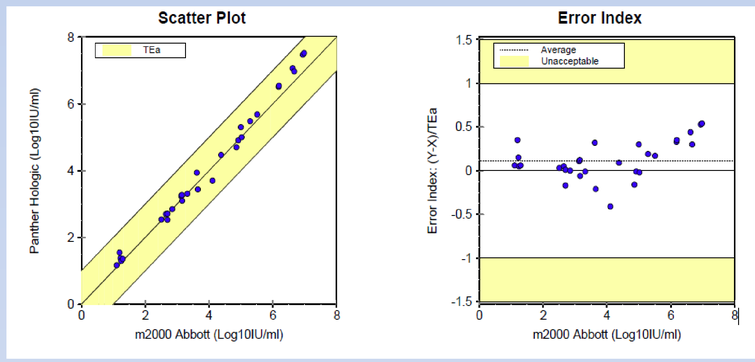

Figure 1: EP Evaluator HCV results for the quantitative comparison of thirty specimens using Abbott m2000 and Panther Hologic methods.

Two Instrument Comparison

X Method m2000 Abbott — Y Method Panther Hologic

Experimental Results

Specimen	X	Y	Error Index	Specimen	X	Y	Error Index	Specimen	X	Y	Error Index
111344	3.16	3.10	-0.60	153890	3.13	3.24	0.11	60579	4.92	4.91	-0.01
120422	5.51	5.68	0.17	157583	6.62	7.06	0.44	62561	4.86	4.70	-0.16
126989	2.70	2.53	-0.17	158505	1.20	1.55	0.35	65274	5.02	5.00	-0.02
128743	6.94	7.47	0.53	159215	5.29	5.48	0.19	65278	4.38	4.47	0.09
136157	3.15	3.27	0.12	160455	2.85	2.85	0.00	68474	3.65	3.44	-0.21
141107	1.11	1.17	0.06	160657	5.00	5.30	0.30	74001	1.30	1.36	0.06
141193	2.65	2.70	0.05	160815	6.98	7.52	0.54	83201	1.23	1.38	0.15
141227	3.32	3.31	-0.01	160821	6.18	6.51	0.33	83204	2.51	2.54	0.03
149879	2.70	2.71	0.01	1932	4.11	3.70	-0.41	88269	6.67	6.97	0.30
149980	6.19	6.54	0.35	44683	1.25	1.30	0.05	91349	3.62	3.94	0.32

Values with an "X" were excluded from the calculations.

Figure 2: Two instrument comparison data in the quantitative study. Accuracy results.

The qualitative performance study showed a 100% of positive agreement and a 95% of negative agreement for an overall agreement of 98.3% between both methods. Only one discrepant result was obtained in a specimen with low viral load that came out negative on the Abbott m2000 instrument and positive on the Panther system. This could be explained because the Aptima HCV Quant Dx Assay has a lower detection limit (<3.9 IU/mL) than the Abbott System (<12 IU/mL) in plasma specimens.

RESULTS CONT.

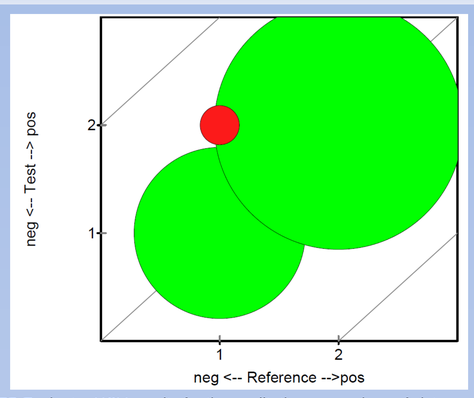

Figure 3: EP Evaluator HCV results for the qualitative comparison of sixty specimens using Abbott m2000 and Panther Hologic methods.

Statistical Summary

	Negative Reference	Positive Reference	Total
Negative Test	19	--	19
Positive Test	1	40	41
Total	20	40	60

Number excluded or missing: 0

Figure 4: Two instrument comparison data in the qualitative study.

Precision

For the precision study, the EP Evaluator results showed a mean of 3.861 $\log_{10}$ IU/mL with a standard deviation of 0.047. This value was within the 2 SD range (3.767-3.954) fulfilling the requirements for repeatability. The coefficient of variation (CV) obtained was 1.2 % (Figure 5).

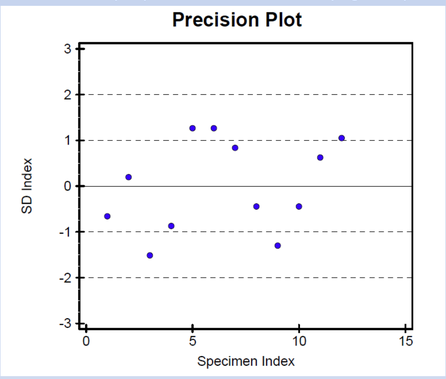

Figure 5: Simple precision experiment data using a "within-run" type of experiment.

Specificity of the Aptima HCV Quant Dx Assay was 100 % in agreement with the results obtained from Abbott m2000 (data no showed).

CONCLUSIONS

- ❑ The results obtained show that the Aptima HCV Quant Dx assay is a highly sensitive, accurate, and reproducible assay with an analytical performance equal to that of the Abbott Realtime HCV assay.
- ❑ The lower detection limit of the Aptima HCV Quant Dx Assay allowed for the detection of a sample with low viral load that came out negative on the Abbott m2000 instrument.
- ❑ The Aptima HCV Quant Dx assay is also a faster and more efficient test than the Abbott Realtime HCV assay, improving productivity. The continuous access, with no batching restrictions, allows for the loading of specimens at any time.
- ❑ This is helpful in the lab setting because it substantially reduces hands-on time needed to set up the test and allows for shorter wait time for results.
- ❑ All of this makes the Aptima HCV Quant Dx assay on the fully automated Panther system an excellent option to detect and quantify HCV and to monitor treatment efficacy.